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# Covalent photolinker-mediated immobilization of an intermediate dextran layer to polymer-coated surfaces for biosensing applications

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**Abstract**

A new method is presented for the covalent binding of dextran as an intermediate layer on surface acoustic wave (SAW) devices. For biosensing applications in aqueous media commercially available SAW devices require surface passivation to prevent corrosion of the aluminum device structures in electrolytes. Thin films of polyimide and parylene revealed exceptional passivation properties. They were used as a base for dextran immobilization. Covalent binding of dextran to polymer-coated surfaces was achieved by photoimmobilization. Aryldiazirine-functionalized bovine serum albumin served as the multifunctional light-activable linking agent (photolinker polymer). Dextran and photolinker polymer were mixed and photobonded to sensor surfaces. Essential photoimmobilization parameters were optimized. The binding of proteins to dextran applying carbodiimide chemistries was exemplified with anti-urease antibodies and the feasibility of specific immunosensing was investigated on SAW sensors connected to a fluid handling system. 

*Keywords:* Biosensor; Dextran; Mass-sensitivity; Photoimmobilization; SAW

## 1. Introduction

In the past decade surface acoustic wave (SAW) sensors have increasingly been considered as appropriate tools for biosensing (Baer et al., 1992; Kondoh et al., 1994; Tom-Moy et al., 1995; Welsch et al., 1996). To create a biosensor, these devices require surface coating with sensing layers which enable specific biological interactions. These interactions are detected by determining changes in surface wave velocity, predominantly caused by mass adsorption or viscosity changes.

In the above-mentioned studies the SAW devices used were self-made and based on special developments. SAW devices used in this study are low-loss filters based on horizontal polarized shear waves (HPSW) with lithiumtantalate as the substrate material (Rapp et al., 1995b; Barié et al., 1998). They are widely used in electronic telecommunication systems and thus are commercially available at low cost. The operating frequency is 380 MHz and the typical attenuation during operation is only 2 dB in air, and 4 dB in water (Rapp et al., 1993). This low attenuation enables the measurement of the sensor signal in the most simple and accurate way by recording changes of the resonance frequency of an oscillator circuit with the device used as the frequency determining element.

For biosensing purposes pretreatment of these SAW device surfaces with passivating layers is essential to protect the metal surface and to guarantee the stability of the aluminum electrodes in electrolyte solutions (Wessa et al., 1998). Polyimide (Sato et al., 1993) and parylene (Williams, 1992) were found to provide exceptional passivation properties, enable serial production and suit subsequent surface bioengineering.

Previously used immobilization methods on these devices (Rapp et al., 1995a; Wessa et al., 1996) are often time and labour consuming and hardly transferable to other applications, because special reactive functional groups are required on either the ligand or the supporting material.

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This study introduces a new approach to produce very versatile SAW biosensor platforms. It is achieved by single step, covalent photobonding of an intermediate dextran layer to passivated sensor surfaces. Surface-bound dextran permits experimentally facile, rapid and reproducible immobilization of almost any receptive molecule with established biomolecule coupling chemistries (O'Shannessy et al., 1992).

Dextran is covalently bound to the shielding layer by means of photoimmobilization (Sigrist et al., 1995). The light-activated photolinker polymer serves as a linker between the passivating layer and dextran. Bovine serum albumin, multiply derivatized with light-sensitive trifluoromethyl-aryldiazirine (T-BSA), was described previously as a photolinker polymer (Sigrist et al., 1992). For surface functionalization T-BSA and dextran are mixed and applied to passivated sensor surfaces. After solvent removal, the coated surfaces are exposed to activating light. Aryldiazirines absorb light of wavelength 350 nm and form highly reactive carbenes, which preferably insert into C–H, C–C, C = C, N–H, O–H, or S–H bonds (Dolder et al., 1990). The high degree of substitution of BSA with photoreagents (6–10 mol diazirine per mol BSA) enables the simultaneous insertion of photogenerated carbenes into both dextran and the passivating layer. Thus covalent coupling of dextran to the surface is achieved. The resulting layer structure is schematically depicted in Fig. 1 with polyimide as surface passivating material.

In view of the optimization of the dextran binding process, biotinylated dextran and carboxymethylated dextran were used in this study. Biotinylated dextran served to quantitate the photoimmobilization step by recording the retention of radiolabelled streptavidin. Carboxymethyl-modified dextran was used as a chemical base for covalent binding of sensing biomolecules. Receptive molecules are covalently immobilized to dextran applying chemistries which confer with the nature of the ligand and the purpose of the immobilization.

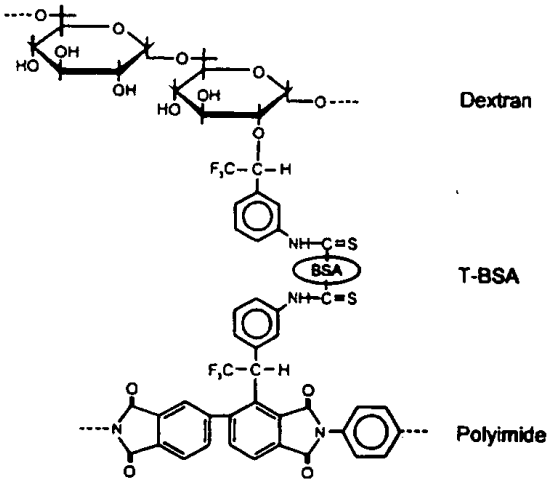


Fig. 1. Scheme for photolinker-polymer-mediated covalent immobilization of dextran to polyimide. The photobonding procedure is not restricted to polyimide.

## 2. Experimental

### 2.1. Materials and instrumentation

Dextrans were obtained from Molecular Probes (Oregon, USA) (D-1856: dextran, biotin, 10 000 MW; D-1953: dextran, carboxymethyl, 70 000 MW). Urease was obtained from Biotrend Chemikalien GmbH (Köln, Germany). Monoclonal anti-urease antibodies, mouse IgG and glucose oxidase were purchased from Sigma–Aldrich Chemie GmbH (Deisenhofen, Germany). N-hydroxysuccinimide (NHS), N-(3-dimethylaminopropyl)-N-ethylcarbodiimide (EDC), Tween 20 and ethanolamine were obtained from Merck KGaA, (Darmstadt, Germany). BSA was purchased from Serva Feinbiochemica GmbH (Heidelberg, Germany). [$^{35}$S]-Streptavidin was obtained from Amersham (Zürich, Switzerland). Polyimide (PI 2610) was a gift of DuPont de Nemours (Bad Homburg, Germany). Dimethyl-aminopropyl-ethoxysilane was purchased from ABCR (Karlsruhe, Germany). T-BSA was synthesized as described by Sigrist (Sigrist et al., 1992). All other reagents were of analytical grade.

Phosphate buffer contained either 0.5, 20 or 50 mM potassium phosphate. The pH was adjusted to 7.2 with KOH. Modified surfaces were washed with 50 mM phosphate buffer containing 0.02% (v/v) Tween 20 (phosphate buffer/Tween). The phosphate buffered saline (PBS) used for immunoreactions consisted of 20 mM phosphate buffer (pH 7.2) containing 140 mM NaCl.

[$^{35}$S]-labelled streptavidin was detected by scintillation counting (Horrocks, 1974) utilizing Ultima Gold (Packard no. 6013329) scintillation fluid (5 ml each sample).

For irradiation a Stratalinker 2400 UV Crosslinker (Stratagene GmbH, Heidelberg, Germany) equipped with five 365 nm (major emission) UV light bulbs (F 158 BL; 15 W black light each) was used. The coated devices were placed 4 cm apart from the light source and illuminated with an irradiance of 0.7 mW cm$^{-2}$.

SAW devices, type SAF380T with an operating frequency of 380 MHz, were purchased from MuRata Company (Japan). The devices are commercially available low-loss-filters based on horizontal polarized shear waves (HPSW, STW) on a 36° rotated $LiTaO_3$ XY cut substrate. For analyte detection modified devices were placed in a round chamber, 8 mm diameter (TO 39). Mounted in teflon adapters the sensors were connected to a fluidic system. The fluidic system consisted of a

peristaltic pump (Ismatec Labortechnik GmbH, Wertheim–Mondfeld, Germany) and an addressable four-port valve (Latek–TMV, Mannheim, Germany). Analyte binding measurements were carried out in an oscillator circuit, which was purchased from the University of Heidelberg (Germany). Resonance frequencies were detected by a Hewlett Packard frequency counter (Hewlett Packard GmbH, Waldbronn, Germany; HP-PM 6680), scanner (HP-PM 2327) and switch (HP-PM 2301). The instruments were controlled by a personal computer via a GPIB interface.

### 2.2. *Methods*

#### 2.2.1. *Surface passivation*

Polyimide shielding layers were covalently attached to the hydrophilic SAW surfaces after silanization (Wessa et al., 1998). For surface silanization, 5 μl dimethyl-aminopropyl-ethoxysilane were dissolved in 9.5 ml methanol and 500 μl water. The solution was stirred for 15 min and was allowed to react overnight. SAW sensors as purchased were dipped in acetone for 10 s and dried by spincoating (3000 rpm, 20 s).

Mounted SAW surfaces were treated with 50 μl silane solution. After 30 s incubation, excess solution was flung off by spinning (30 s, 3000 rpm). The polyimide used for coating purposes consisted of the monomers p-phenylene-diamine and 3,3',4,4'-biphenyltetracarboxylic dianhydride. A mixture of both monomers was diluted with N-methyl-pyrrolidone to a concentration of 0.9 g/ml. Silanized SAW sensors were coated with 50 μl of the monomer mixture for 70 s. Excess was removed by spinning (30 s, 5000 rpm) and the sensors were tempered for 30 min at 473 K and 30 min at 623 K. After this treatment the devices were allowed to cool to room temperature overnight.

In a second series of experiments parylene C (poly-(2-chloro-p-xylylene) was used as a passivating agent. The parylene polymer was deposited on either diced flat glass (5 × 5 mm) or SAW devices in a factory setup by Cometec SA (La Chaux-de-Fonds, Switzerland). The thickness of the parylene film was 0.8 ± 0.3 μm. Parylene-coated diced glass was used for process optimization. SAW devices coated either with parylene or polyimide were used for surface bioengineering and biosensing.

#### 2.2.2. *Immobilization of dextran*

T-BSA and dextran were mixed at indicated ratios in phosphate buffer (pH 7.2). Passivated surfaces were coated with the T-BSA/dextran solution (glass substrate: 20 μl; SAW device: 5 μl), dried under reduced pressure for 2 h at ambient temperature and irradiated for the indicated length of time with the Stratalinker light source. After photobonding, the surfaces were washed repeatedly in chaotropic/detergent containing solutions, allowing 5 min incubation at room temperature for each wash cycle. At least three supports were modified identically, and triplicate samples were analysed by liquid scintillation counting (glass supports) or by recording the change in resonance frequency (SAW device). Mean values were determined and used for further calculations. Identically treated non-irradiated samples served as controls.

#### 2.2.3. *Optimization of surface coating with dextran*

For optimization of the coating procedure, parylene-coated glass substrates were modified with biotinylated dextran as described above. An aqueous solution of [$^{35}$S]-streptavidin (20 μl) was applied to the coated surface and incubated at room temperature for 1 h. After washing with phosphate buffer/Tween (five times) and water (three times) bound [$^{35}$S]-streptavidin was quantified by liquid scintillation counting.

#### 2.2.4. *Protein immobilization on dextran*

For target molecule detection SAW devices were first passivated with parylene or polyimide and coated with carboxymethyl dextran as described above. Aqueous solutions of NHS (100 mM) and EDC (400 mM) were mixed, 5 μl reagent mixture was applied on the pretreated sensor surface and incubated for 7 min. After activation of the dextran carboxyl functions, 10 μl of protein solution (in this case: monoclonal anti-urease antibodies) in 10 mM acetate buffer (pH 5.0) was applied and incubated at room temperature for 20 min. Excess NHS ester was deactivated with 10 μl 1 M ethanolamine. Unbound protein was washed off by repeated rinsing of the sensor surface with 50 mM phosphate buffer (pH 7.2). Non-specific binding sites were saturated with bovine serum albumin (4% w/v in 20 mM phosphate buffer, 10 min, ambient temperature). After a final washing step with 50 mM phosphate buffer, the sensor was ready for biosensing.

#### 2.2.5. *Monitoring of the immunoreaction*

Coated SAW devices were mounted in the flow system and rinsed with PBS until the resonance frequency stabilized (flow rate: 100 μl/min). Solutions containing the antigen or non-specifically interacting (immuno)-reagents, dissolved in PBS were injected in the carrier buffer. Time-dependent resonance frequency changes were monitored under constant flow conditions.

## 3. Results and discussion

### 3.1. *Optimization of the dextran immobilization*

Photobonding of dextran to polymer passivated surfaces was optimized for high protein surface densities. On the one hand the extent of surface coating may

depend directly on the amount of immobilized dextran, on the other hand steric factors are expected to effect protein binding. Since dextran is known to form swollen three-dimensional hydrogels on surfaces, it is possible to bind proteins also to interior binding sites of this matrix, if there is no steric hindrance. For this reason, the optimization was done with biotinylated dextran and [$^{35}$S]-streptavidin and not, directly and more simply, by quantifying radioactive-labelled dextran, which would disregard the steric factor. This assay mimics the general process of protein binding to hydrogels, but in comparison to carbodiimide-based binding procedures it has the advantage of being more rapid, and excluding sources of error due to following modification steps. The multivalency of streptavidin could be a disadvantage of this assay because of possible crosslinking effects, but the results (good reproducibilities, small standard deviations) prove that this method is suitable for optimization purposes.

For optimization purposes it is only necessary to compare the measured radioactive decompositions relatively in one measuring series, hence, the obtained values of dpm (decomposition per minute) were not converted into absolute amounts of streptavidin, but directly compared with each other (see Figs. 2 and 3). The higher the measured signal (dpm), the more streptavidin was bound and the better the coating procedure.

### 3.1.1. Optimization of dextran to T-BSA ratio

Covalent coupling of dextran to passivated sensor surfaces was achieved by T-BSA as a multifunctional light-activable linking agent. The dextran to T-BSA ratio was optimized by treatment of parylene-coated glass substrates with mixtures of biotinylated dextran and T-BSA. Fig. 2 shows increasing streptavidin binding with increasing T-BSA content on the surface, which is attributed to improved dextran immobilization. In the range of 70 to 85 mol% T-BSA (equivalent to 30 and 15 mol% dextran) maximum binding is reached. At high T-BSA contents streptavidin binding dropped drastically in line with the biotinylated dextran content.

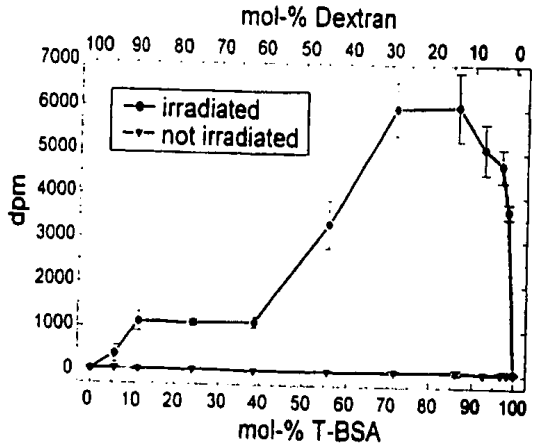


Fig. 2. Optimization of dextran to T-BSA ratio. Parylene-passivated glass substrates were coated with mixtures of biotinylated dextran and T-BSA as indicated and exposed to activating light for 45 min. Control samples were not irradiated. Biotinylated dextran binding was determined by measuring the radioactivity after incubation of modified surfaces with [$^{35}$S]-streptavidin.

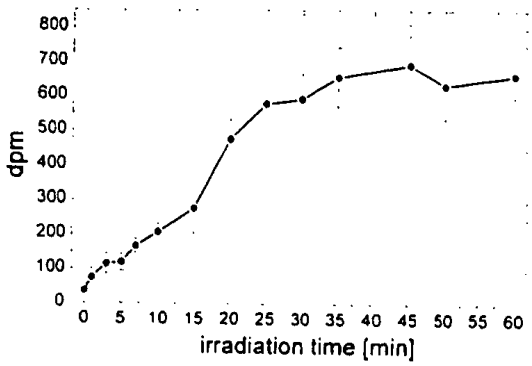


Fig. 3. Optimization of irradiation time. Parylene-passivated glass substrates were coated with a mixture of 25 mol% biotinylated dextran and 75 mol% T-BSA and exposed to light for the indicated length of time.

Non-irradiated, identically treated samples showed very low signals. The latter observation substantiates the light-dependence of the immobilization reaction.

### 3.1.2. Optimization of irradiation time

As shown in Fig. 2, dextran immobilization strictly depends on light activation. In order to optimize the time of irradiation, parylene-treated glass substrates were coated with 25 mol% biotinylated dextran and 75 mol% T-BSA, dried and exposed to light for varying time lengths. After incubation with [$^{35}$S]-streptavidin and extensive washing of the modified surfaces, retained radioactivity was determined by liquid scintillation counting. The results are summarized in Fig. 3. The extent of dextran binding saturated at 45 min irradiation. No additional binding of dextran was achieved with prolonged light exposure.

### 3.1.3. Reproducibility and surface density of immobilized dextran

The reproducibility of the coating procedure was determined by coating of 10 parylene-coated glass sheets under identical conditions, treating with [$^{35}$S]-streptavidin and measuring the radioactive decompositions. A coefficient of variation of 8% was found.

After photoimmobilization under optimized conditions 7% of the applied dextran was recovered on the surface. This corresponds to a surface density of 1 ng/mm$^2$ dextran.

### 3.2. Protein immobilization and immunosensing

The covalently bound dextran layer enabled versatile, fast and fluid system supported surface modification. It has been described by Bayer & Wilchek (1980); Wilchek & Bayer (1984, 1988) that the reaction of biotin-modified substrates with avidin or streptavidin leads to a universal surface for immobilization of a wide range of biotinylated ligands. O'Shannessy et al. (1992); Johnsson et al. (1991, 1995); Löfas et al. (1993) describe biomolecule immobilization approaches, in which carboxymethyl-modified dextran surfaces are converted to reactive NHS esters, amines, hydrazines or sulfhydryls. This study provides evidence for the use of a photobonded dextran layer as the basis for monoclonal antibody immobilization and subsequent specific antigen detection on SAW sensors.

#### 3.2.1. Immobilization of monoclonal antibodies on SAW devices

Immobilization of monoclonal anti-urease antibodies using carbodiimide chemistries is shown in Fig. 4. All immobilization reaction steps were monitored by the frequency response of the SAW sensor: the carboxymethylated dextran-coated SAW device (shielding layer: polyimide; operating frequency: 379.43 MHz; insertion loss: 4.89 dB) was rinsed with the carrier buffer (20 mM phosphate buffer) until a stable base line was obtained. A mixture of NHS and EDC was injected to activate the carboxymethylated dextran. Due to different conductivities of both solutions (buffer and EDC/NHS) a sensor response was observed. Subsequently, the protein solution was injected. The resonance frequency decreased indicating covalent antibody binding. In a final step ethanolamine was injected to cap remaining NHS ester functions, and non-specific protein binding sites in the system were blocked with BSA.

#### 3.2.2. Specific and non-specific binding to photobonded dextran surfaces

To distinguish between specific and unspecific sensor responses, polyimide/carboxymethylated dextran-coated SAW devices were modified with monoclonal anti-urease antibodies. Fig. 5 shows the immunoreaction with urease and non-specifically interacting analytes.

To observe the immunoreaction of immobilized antibodies with the corresponding antigen, a solution of urease (500 μg/ml in PBS) was injected in the carrier buffer. Subsequent rinsing with PBS slightly reversed the signal (release of loosely adsorbed urease), leaving a net frequency shift of 110 kHz, representing the irreversible specific binding of urease to the antibodies.

The reagents non-complementary to monoclonal anti-urease led to small and nearly reversible frequency shifts. In addition, a SAW device coated with polyimide and carboxymethylated dextran but without immobilized antibodies was treated with urease. The sensor response is shown in Fig. 5 as dotted line.

These minor effects are due to non-specific adsorption of either reagent applied. In contrast, the specific reaction of urease led to a significant and irreversible frequency shift.

The high concentrations of urease and non-specific antigens (500 μg/ml) were used in first experiments to obtain high sensor signals with surplus reagents. New experiments show the same results with lower concentrations (15 and 65 μg/ml, respectively). (Data not shown.)

#### 3.2.3. Reproducibility of sensor responses

The reproducibility of sensor responses of the dextran-coated SAW sensors depends on several parameters. One important parameter is the reproducibility of the immob-

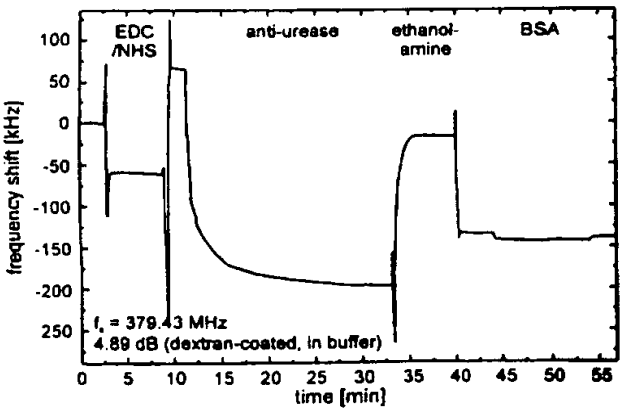


Fig. 4. Sensor response during the immobilization of monoclonal anti-urease antibodies on carboxymethyl-modified dextran. The pristine sensor surface was passivated with polyimide and the antibody was immobilized by reacting with in situ generated NHS esters.

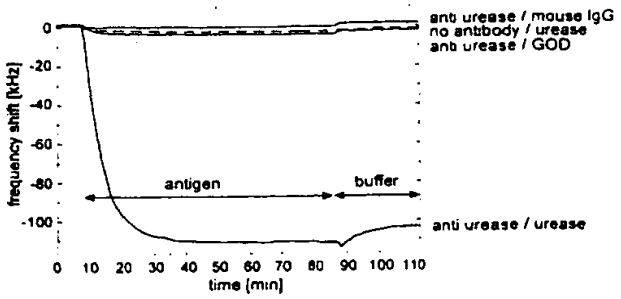


Fig. 5. Sensor responses of identically prepared sensors (coated with monoclonal antibodies to urease on carboxymethylated dextran) to specific (urease) and non-specific (mouse IgG, glucose oxidase) antigens. In addition, the response of a dextran-coated sensor (without immobilized antibodies) to urease is shown.

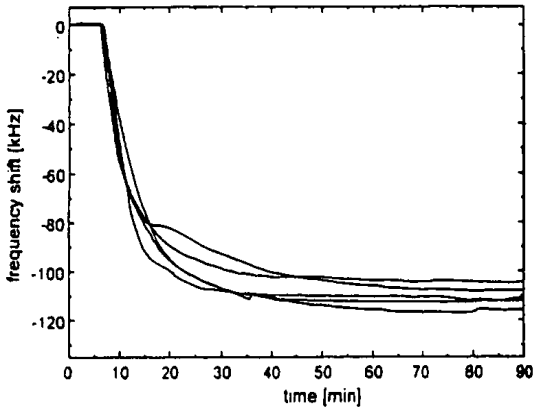


Fig. 6. Reproducibility of sensor responses of dextran-coated SAW devices with immobilized monoclonal anti-urease antibodies to immunoreactions with urease.

ilization of dextran. As mentioned above, this immobilization step showed a coefficient of variation of 8%.

For studying the reproducibility of sensor signals due to immunoreactions, five dextran-coated SAW devices were modified with monoclonal anti-urease antibodies and treated with a solution of urease (100 μg/ml in PBS). Fig. 6 shows the sensor responses during immunoreaction. A mean frequency shift of 110 kHz was obtained with a coefficient of variation of 12%.

## 4. Summary and conclusion

A new method for the coating of sensor surfaces with a dextran layer is presented. Covalent photobonding of dextran to polymer passivated surfaces was achieved by photolinker-mediated immobilization with aryldiazirine-functionalized bovine serum albumin. Process parameters were optimized for maximum protein binding. The optimized dextran coating solution consists of 25 mol% dextran and 75 mol% T-BSA. The photoreaction was completed after 45 min irradiation. The use of dextran as an intermediate layer for biosensor applications was exemplified by immobilization of monoclonal anti-urease antibodies on carboxymethylated dextran. Thus, the engineering of a very versatile SAW biosensor has been attained. The method is applicable for simple, rapid and reproducible immobilization and detection of biomolecules.

## References

Baer, R.L., Flory, C.A., Tom-Moy, M., Solomon, D.S., 1992. STW chemical sensors. In: Proceedings of the IEEE Ultrasonics Symposium, Tucson, AZ, USA, pp. 293–298.

Barié, N., Rapp, M., Ache, H.J., 1998. UV crosslinked polysiloxanes as new coating materials for SAW devices with high longterm stability. Sens. Act. B 46, 97–103.

Bayer, E.A., Wilchek, M., 1980. The use of the avidin–biotin complex as a tool in molecular biology. Methods Biochem. Anal. 26, 1–45.

Dolder, M., Michel, H., Sigrist, H., 1990. 3-(Trifluoromethyl)-3-(m-isothiocyanophenyl)diazirine, synthesis and chemical characterization of a heterobifunctional carbene-generating crosslinking reagent. J. Prot. Chem. 9, 407–415.

Horrocks, D.L., 1974. Applications of Liquid Scintillation Counting. Academic Press, New York.

Johnsson, B., Löfas, S., Lindquist, G., 1991. Immobilization of proteins to a carboxymethyldextran-modified gold surface for biospecific interaction analysis in surface plasmon resonance sensors. Anal. Biochem. 198, 268–277.

Johnsson, B., Löfas, S., Lindquist, G., Edstrom, A., Müller-Hillgren, R.M., Hansson, A., 1995. Comparison of methods for immobilization to carboxymethyl dextran sensor surfaces by analysis of the specific activity of monoclonal antibodies. J. Mol. Recogn. 125–131.

Kondoh, J., Matsui, Y., Shiokawa, S., Wlodarski, W., 1994. Enzyme immobilized SH-SAW biosensor. Sens. & Act. B20, 199–203.

Löfas, S., Johnsson, B., Tegendal, K., Rönnberg, I., 1993. Dextran modified gold surfaces for surface plasmon resonance sensors: immunoreactivity of immobilized antibodies and antibody-surface interaction studies. Colloids & Surfaces B: Biointerfaces 1, 83–89.

O'Shannessy, D.J., Brigham-Burke, M., Peck, K., 1992. Immobilization chemistries suitable for use in the BIAcore surface plasmon resonance detector. Anal. Biochem. 205, 132–136.

Rapp, M., Moss, D.A., Wessa, T., Reichert, J., Ache, H.J., 1993. Mass-sensitive immunosensor based on surface acoustic wave devices for in-situ measurements in water. Presented at the Eurosensor VII, Budapest, Hungary.

Rapp, M., Wessa, T., Ache, H.J., 1995a. Modification of commercially available low loss SAW devices towards an immunosensor for in-situ measurements in water. IEEE Symposium, Seattle.

Rapp, M., Barié, N., Stier, S., Ache, H.J., 1995b. Optimization of an analytical SAW microsystem for organic gas detection. In: Proceedings of the IEEE Ultrasonics Symposium, Seattle, pp. 477–480.

Sato, M., Yamamoto, T., Takeuchi, M., Yamanouchi, K., 1993. Humidity sensitivity of lamb waves on composite polyimide/ZnO/$Si_3N_4$ structure. Jpn. J. Appl. Phys. 32, 2380–2.

Sigrist, H., Gao, H., Wegmüller, B., 1992. Light-dependent, covalent immobilization of biomolecules on inert surfaces. Bio/Technology 10, 1026–1028.

Sigrist, H., Collioud, A., Clémence, J.F., Gao, H., Luginbühl, R., Sänger, M., Sundarababu, G., 1995. Surface immobilization of biomolecules by light. Opt. Engng 34, 2339–2348.

Tom-Moy, M., Baer, R.L., Solomon, D.S., Doherty, T.P., 1995. Atrazine measurements using surface transverse wave devices. Anal. Chem. 67, 1510–1516.

Welsch, W., Klein, C., Schickfus, M., Hunklinger, S., 1996. Development of a surface acoustic wave immunosensor. Anal. Chem. 68, 2000–2004.

Wessa, T., Rapp, M., Sigrist, H., Ache, H.J., 1996. Immunosensing with surface acoustic wave devices using photoinduced immobilization techniques. Presented at the Biosensor, Bangkok.

Wessa, T., Barié, N., Rapp, M., Ache, H.J., 1998. Polyimide, a new shielding layer for sensor applications. Sens. & Act. (in press).

Wilchek, M., Bayer, E.A., 1984. The avidin–biotin complex in immunology (Review). Immunol. Today 5, 39–43.

Wilchek, M., Bayer, E.A., 1988. The avidin–biotin complex in bioanalytical applications (Review). Anal. Biochem. 171, 1–32.

Williams, D.F., 1992. Materials science and technology. Journal title please 14, 317–361.